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ABSTRACT

The curriculum guide for teaching science to gifted intermediate grade students presents material to be used for a unit on microbiology, as well as suggestions for a second unit on the subject. Examined in the unit are the structures, functions, growth, development, uses, and environments of different kinds of microorganisms, with an emphasis on bacteria. The first section of the guide, intended for teachers, presents suggested instructional approaches for teaching microbiological concepts and covers both the range of subject matter content and behavioral objectives. The second section, Suggested Learning Activities, is addressed to the student and contains four sample lessons. The third section, meant to be used by both teacher and student, defines scientific and technical terms, presents certain aspects of the classification of microorganisms, and provides directions for 10 technical procedures used in the projects suggested in the guide. Also listed are some resources and references, and recommendations concerning further study in microbiology. (KW)

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science

A UNIT ON MICROBIOLOGY

CURRICULUM GUIDE FOR TEACHING GIFTED CHILDREN SCIENCE IN GRADES FOUR THROUGH SIX

CALIFORNIA STATE DEPARTMENT OF EDUCATION
Max Rafferty — Superintendent of Public Instruction • Sacramento, 1970

science

A UNIT ON MICROBIOLOGY

CURRICULUM GUIDE FOR TEACHING GIFTED CHILDREN SCIENCE IN GRADES FOUR THROUGH SIX

Prepared for the
DIVISION OF SPECIAL EDUCATION
California State Department of Education

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U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
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Foreword

California public schools are charged with providing ample opportunity for every girl and boy of school age to become knowledgeable in the basic subjects and proficient in using the basic skills of learning. It is equally important that the educational programs offered by the schools be of sufficient scope and depth to permit each child to learn at the rate and to the full level that his ability permits.

In conducting their educational programs, the schools must adopt practices that are flexible enough to allow for whatever adjustments are required to meet each pupil's need of special education. The talented are among those for whom such adjustments are necessary. Recently the State Department of Education directed and coordinated a federally funded project for the development of curriculum materials of the type needed for the education of gifted children. The materials, which reflect the best thinking of people who are well qualified both by education and by experience, are innovative and professional.

This curriculum guide, one of a series, is concerned with the teaching of science to mentally gifted pupils in grades four through six. The concepts and suggestions contained in it merit thoughtful attention, appropriate interpretation, and wise application.

Max Rafferty

Superintendent of Public Instruction

Preface

This curriculum guide, which was planned and completed as part of a project under provisions of the Elementary and Secondary Education Act, Title V, is intended to assist teachers of mentally gifted children whose general mental ability is in the top 2 percent of all girls and boys.

Curriculum Guide for Teaching Gifted Children Science in Grades Four Through Six presents material that can be used for a unit on microbiology, as well as suggestions for a second unit on the subject. Numerous learning activities are proposed with the view to their serving as springboards to other ideas and other experiences.

This publication is one of a series of curriculum guides designed for the following educational levels: grades one through three, four through six, seven and eight, and nine through twelve. The guides were prepared under the direction of John C. Gowan, Professor of Education, and his assistant, Joyce Sonntag, Assistant Professor of Education, both of San Fernando Valley State College.

A curriculum framework that is designed for use in developing programs for mentally gifted minors was also developed in the project. This framework was prepared under the direction of Mary N. Meeker, Associate Professor of Education, University of Southern California, and James Magary, Associate Professor of Educational Psychology, University of Southern California.

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INTRODUCTION

This curriculum publication, prepared for the guidance of gifted pupils in grades four through six, explores a specific branch of natural science – that of microbiology, which can be defined quite simply as the study of microscopic living things. The structures, functions, growth, development, uses, and environments of different kinds of microorganisms, with a major emphasis on bacteria, are examined in this unit; and many learning activities are proposed.

The body of the guide is divided into three distinct sections. The first is written for the benefit of the teacher. Entitled "Suggested Instructional Approaches," it provides information and suggestions for the introduction, development, application, and extension of microbiological concepts. Behavioral objectives in terms of the learner are stated, and the range of subject-matter content is discussed. Seven proposed phases of instruction are offered to the teacher.

Section II, "Suggested Learning Activities," is addressed to the pupils and contains four sample lessons on microbiology. The activities described in this part are related to the instructional approaches in Section I. It is important to note that these lessons are not to be treated as blocks of learning, nor are they to be restricted by daily time boundaries; rather, they should be made part of a continuous flow of learning opportunities and should be kept flexible enough so that each pupil can progress (1) at the rate of which he is capable; and (2) to the extent of his interest, desire, and motivation.

The third section, "Technical Terms and Procedures," is meant to be used both by the teacher and by the learners. Containing much detailed information, this part lists definitions of scientific and technical terms, presents certain aspects of the classification of microorganisms, and makes available ten sets of directions for technical procedures that can be used in the experiments and projects proposed by the guide. These procedures relate directly to the content of sections I and II.

The concisely stated yet abundant information in Section III serves as a source supply of technical aids and data for the users of the guide. Wall charts showing the ten procedures can be set up in the classroom, or copies of the procedures – in booklet form or in sets of sheets – can be distributed individually to the pupils. Methods of utilizing these procedures are at the option of the teacher. Of special value to this unit is the unnumbered section, "Resources and References," which appears toward the back of the guide. This part contains a list of supplies needed for the suggested experiments and projects, a brief list of firms and agencies from which materials and equipment can be obtained, a recommended reading list for the gifted learners, and a list of professional references.

At the end of the guide is an Addendum that contains brief guidelines and recommendations concerning further study in the field of microbiology. Its purpose is to suggest one of several possible exploratory avenues which may be taken by those pupils who want to learn more about microorganisms.

Particular mention should be made of the flow chart that accompanies this guide. Designed for teacher and pupil use, it shows the scope and sequence of the content of the unit as proposed in the guide. The chart is keyed to indicate the movement of the class from total-group study to small-group study to individual study. Specific areas of research and investigation are identified on the chart, with Roman numerals to set them off and arrows to denote their interaction.

Most of the flow chart is contained within a line-drawn hexagon-like enclosure. This enclosure is not meant to be a "boundary" of any sort; rather, it is intended simply to remind the teacher and the learners that there should be constant, continuing class interaction and evaluation of all small-group and individual activities.

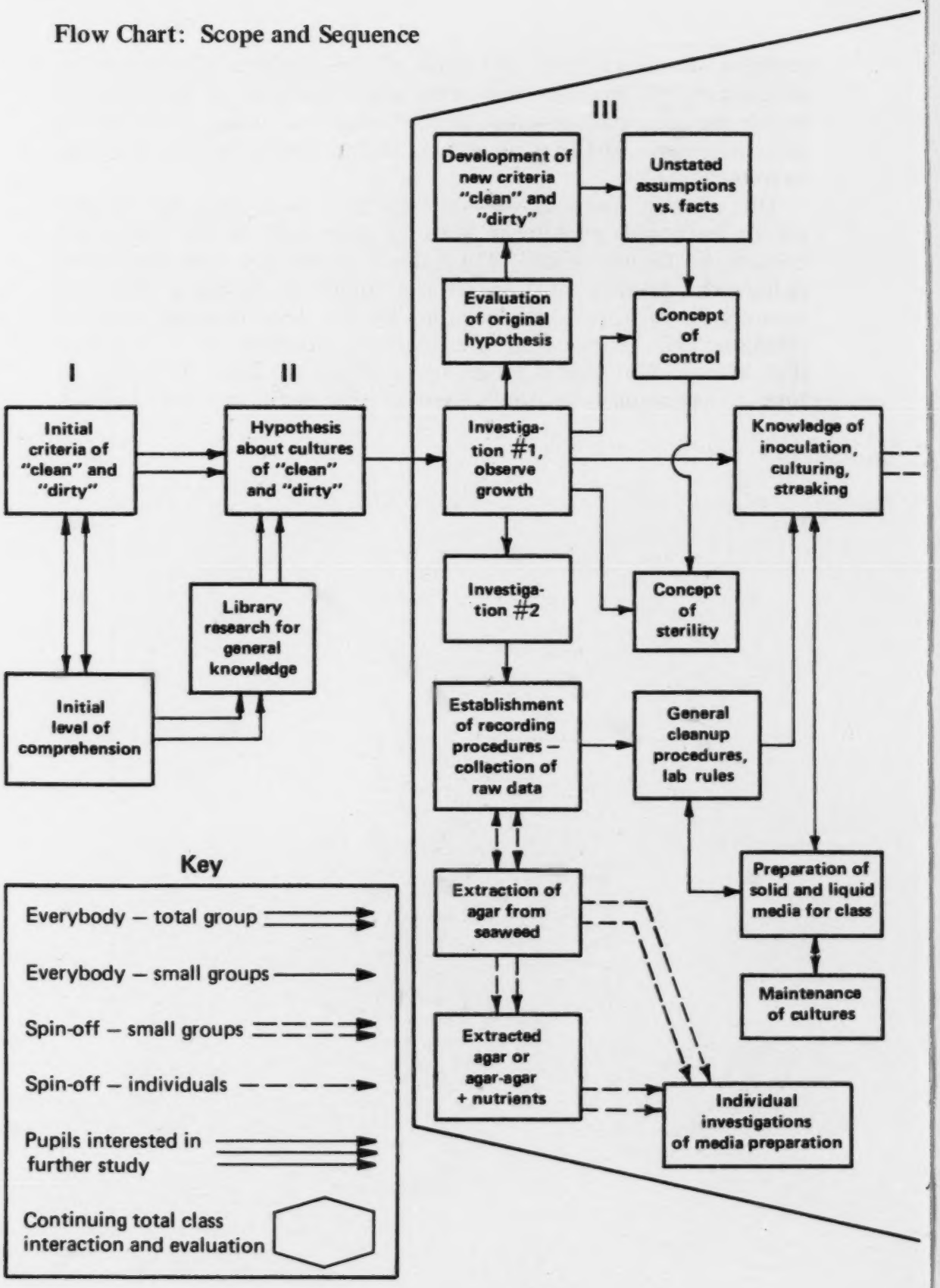
The chart shows a consecutive total movement from area I to the gateway of area VII. It is important to bear in mind, however, that some pupils may be participating in spin-off activities instead of moving through the total sequence. The entire sequence is achieved for all the learners by means of (1) sharing findings and results for the benefit of everyone; (2) holding class discussions; and (3) making class evaluations.

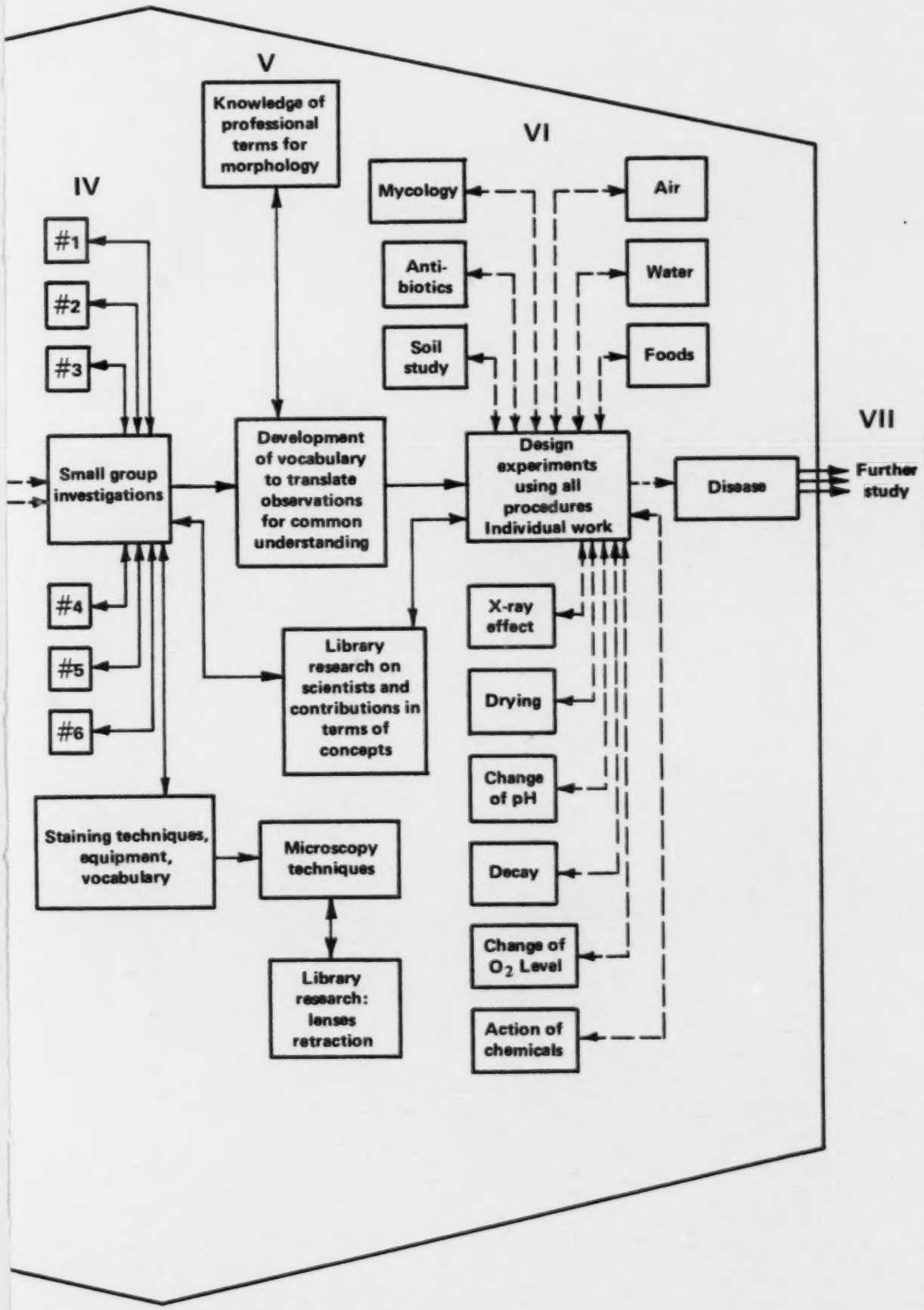
This guide on microbiology is possible for the gifted child because he possesses the extraordinary qualities of persistence and curiosity – qualities that will enable him to follow through the rather lengthy series of procedures and investigations involved in the unit. His ability to take large masses of raw data, detailed procedures, and

complex library research and from all this plethora of information to perceive relationships and make generalizations on his own will enable him to cope with this special branch of biology in the depth presented here. Children of average ability might find the material overwhelming.

The essential purpose of the guide is to encourage the individual to learn and explore at his own pace and to the depth and breadth he desires while he maintains interaction with the entire group. The teacher must decide how much to unfold in the class sessions on microbiology, according to the time modules that are arranged and followed in the teaching situation. It is assumed that in any educational program for gifted children, flexibility of time is determined by the nature of the work, not by the bell.

Flow Chart: Scope and Sequence


I
II
III
Initial criteria of "clean" and "dirty"
Hypothesis about cultures of "clean" and "dirty"
Library research for general knowledge
Initial level of comprehension
Investiga-tion #1, observe growth
Investiga-tion #2
Development of new criteria "clean" and "dirty"
Unstated assumptions vs. facts
Evaluation of original hypothesis
Concept of control
Concept of sterility
Knowledge of inoculation, culturing, streaking
Establishment of recording procedures – collection of raw data
General cleanup procedures, lab rules
Extraction of agar from seaweed
Preparation of solid and liquid media for class
Maintenance of cultures
Extracted agar or agar-agar + nutrients
Individual investigations of media preparation
Key
Everybody – total group
Everybody – small groups
Spin-off – small groups
Spin-off – individuals
Pupils interested in further study
Continuing total class interaction and evaluation


V
Knowledge of professional terms for morphology
VI
IV
Mycology
Air
#1
Anti-biotics
Water
#2
Soil study
Foods
#3
VII
Small group investigations
Development of vocabulary to translate observations for common understanding
Design experiments using all procedures Individual work
Disease
Further study
#4
X-ray effect
Library research on scientists and contributions in terms of concepts
#5
Drying
#6
Change of pH
Staining techniques, equipment, vocabulary
Microscopy techniques
Decay
Change of O_2 Level
Library research: lenses retraction
Action of chemicals

Section I

SUGGESTED INSTRUCTIONAL APPROACHES

This section, addressed to the teacher, suggests ways of helping gifted pupils in grades four through six to learn about microbiology and to apply what they have learned in experiments, projects, and other activities. Both general and specific concepts and objectives pertaining to the unit are listed; pupil techniques are recommended; the scope of the subject matter is discussed; basic information considered helpful to the teacher is included; and seven phases of instruction are presented.

Concepts and Objectives

As the children proceed through their learning experiences, they should be able to grasp the general, overall concept for this unit on microbiology and to attain the general objectives supporting that concept.

General Concept

Living things are interdependent with one another and with their environment.

General Objectives

The learner will:

1. Gain background knowledge, through research and discussion, of basic principles or generalizations in the field of microbiology.
2. Translate his knowledge into the posing of specific problems for investigation.
3. Recognize the nature of the *hypothesis* and be able to formulate specific hypotheses.
4. Master the equipment and the procedural tools necessary to conduct investigations or experiments.
5. Develop the terminology or vocabulary necessary for use in the investigations.

6. Design investigations by which he can collect evidence to support each hypothesis.
7. Conduct the investigations that he has designed.
8. Establish valid criteria for accepting or rejecting each hypothesis.
9. Evaluate his evidence and draw conclusions based on his evaluation.

The specific concepts and objectives of this unit on microbiology are those with which the children should be directly and actively involved. If specifics are not mastered or at least experienced, generalizations will not likely be understood or appreciated.

Specific Concepts

1. *Microorganisms exist almost everywhere.*
2. *The production and growth of microorganisms can be altered by changing the environments of the organisms.*

Specific Objectives

The learner will:

1. Observe the existence of microorganisms through controlled experimentation.
2. Develop and use the tools of inoculation, culturing, staining, microscopy, and classification.
3. Provide environments suitable for the growth of simple organisms.
4. Provide environments suitable for the growth of visible colonies of bacteria.
5. Change the environment of bacteria in certain specific ways; for example, by temperature, by nutrition, by radiation, and by moisture content to ascertain the kind of environment necessary for growth, per se, as well as the kind of environment that provides relative kinds of growth.

Pupil Techniques

In this unit the children should be helped to learn the techniques of professional bacteriologists – techniques that are necessary for accomplishing objectives in the following kinds of activity:

1. The use of research information
2. The recording of data
3. The use of controls in experimentation
4. Technical procedures

5. Validation of experiments
6. Communication and interaction with other persons
7. Evaluation of results through the use of established criteria

General Remarks

Microorganisms exist nearly everywhere. Microorganisms, also called bacteria, can be found in the ice of Antarctica, as well as in the bubbling geysers of Yellowstone National Park. They exist liberally on the hands, the teeth, and the hair of human beings and animals. They cover tables and floors. Because air, water, and soil abound with these living things, it is sometimes convenient to divide the study of microorganisms into three areas of concentration: (1) microscopic life that exists in the air; (2) that which exists in the water; and (3) that which exists in the soil.

A further classification of microorganisms is concerned with three types of living things that can be found in air, water, and soil: molds, yeasts, and bacteria. Molds (multicellular) and yeasts (unicellular) belong to the plant group known as fungi, while bacteria are frequently referred to as "germs." It is difficult to study bacteria, however, without encountering molds. The study of fungi, including molds, can be approached from the viewpoint of a distinct branch of biology called mycology. While the general techniques in the present unit are appropriate tools for mycologists, a different kind of nutrient medium and a different temperature of incubation are used for the purposes of this guide. (If you care to pursue the study of mycology with your pupils, a list of resource materials may be found at the back of the guide under "Resources and References.")

This study of microbiology for gifted children in the fourth, fifth, and sixth grades has been designed to develop and to use the tools of inoculation, culturing, staining, microscopy, and classification for the study of bacteria. Through the use of these tools, the children will learn of the existence of microorganisms and their distribution; they will learn about the morphology of bacteria; they will learn the kinds of environments necessary for the growth of bacteria; and they will learn possible modes of transmission of microorganisms.

The pupils will be able to provide suitable environments wherein single organisms can grow luxuriantly and can produce themselves in abundance so that they will become colonies that are visible to the human eye. The children will also be able to change the environment of the bacteria to demonstrate that unless the organisms can adapt to the change, they cannot continue to exist.

The techniques of the professional bacteriologist will be taught in order that the gifted learners can be successful in bringing the invisible world of microbes to a visible state.

Disease-producing organisms will not be studied in this unit. It is important, however, to stress that the techniques to be used during the entire unit are the same as those that would be used in studying, investigating, and dealing with organisms that produce or transmit disease. Just as much care in handling the equipment should be taken when studying the organisms referred to in this guide as would be taken if the organisms were disease producers. With this kind of approach to the handling and disposition of all related materials, the content of the unit is entirely safe for children to study and investigate.

Phase One: Development of Background Knowledge

Appropriate background knowledge is essential to a study of the type suggested by this guide. The gifted pupils should be reasonably well prepared for the work they will be doing when the unit gets under way.

Objective

The pupil will develop, through discussion and research, the background knowledge that is considered necessary for this study of bacteria.

You might ask the children questions such as the following:

- Why do foods rot?
- What makes cheese?
- Are you a microbe carrier?
- What makes microorganisms "bad guys"?
- Are *all* microorganisms "bad guys"? If so, how?
- How would you go about finding information on microorganisms?

The answers to questions like these will help you to determine quickly the pupils' levels of comprehension with respect to the subject of microbiology or bacteriology. Try to get the children to recall whatever knowledge that they have about bacteria.

Now consult the reading list for pupils under "Resources and References" at the back of this guide. Have the children read those books on the list that relate to the topics suggested in Lesson One in the pupils' section (Section II). Also, ask them to read articles in encyclopedias concerning these topics. Then arrange for individual

and group reports and class discussions about the books and articles that have been read.

Phase Two: Introduction of Materials

In this phase, introduce to the children some of the materials they will be using. Bring out the idea of sterility in the context of bacteriology.

Objectives

1. *The pupil will use a petri dish and agar.*
2. *The pupil will define sterility in terms of microbiology.*

Introduce a plastic petri dish that has nutrient agar media on the bottom of it. (See Section III for preparation procedures.) Because it is sterile, the dish should not be opened until you are ready to use it.

Ask the learners what "sterile" means. Ask them why the petri dish should not be opened. (Answer: Because it would allow organisms from the air to enter; therefore, the surface would no longer be sterile.)

Ask the pupils what the material on the bottom of the dish resembles. (Answer: Lemon gelatin, although the material looks firmer.) What is it called? (Answer: Agar.) Where does agar come from? (Answer: From certain species of seaweed. It is prepared commercially by companies that grow beds of seaweed on the ocean floor. It is collected by divers, washed thoroughly, and pressured in huge pressure cookers for several days. The extract from the pressured material is then poured into large flat tanks and allowed to cool. At about 45° C., the liquid becomes solid. The water and the solid impurities drop out. The solid agar is then reheated; under pressure the moisture is withdrawn, and a powder remains. This powdered agar is then sold commercially by the pound. When it is mixed with water, boiled, and allowed to cool, it returns to its original form.)

Phase Three: Development and Application of a Concept

The activities proposed in this phase, along with the instruction and guidance suggested, are intended to help the children understand quite clearly that many living things in this world are invisible to people without the use of magnifying equipment – that life is not just what one sees about him.

Concept

Living things are not always visible to the naked human eye.

Objectives

1. *The pupils will "grow" microorganisms from their own hands to produce visible colonies on the surface of the agar.*
2. *The pupils will synthesize criteria of "clean" based on their own knowledge and understanding of the term. They will synthesize criteria of "dirty" in the same manner.*

Raise questions such as the following: What would happen if a fingerprint were left on the surface of the agar? (Answer: After 24 hours a growth of bacterial colonies would appear.) What would happen if an insect were allowed to walk across the surface of the dish? What would happen if the lid were left off the plate for ten minutes? What would happen if rainwater fell on the plate?

The children, as a group, should hold brainstorming sessions in which they bring up "what if" questions to investigate at a later time if they wish to do so.

Ask the learners to look at their hands. Are their hands dirty? Are their hands clean? What does "clean" mean? What does "dirty" mean? Are their fingernails clean or dirty? Can dirty hands be made clean by washing just with water? What if they use soap also? Can the children define the terms "clean" and "dirty"?

The entire class can now be divided into groups of five or six pupils each. (During this experiment and all others in this unit, it is important that the children abide by the following rules: (1) the tabletops used should be washed with disinfectant after each session; (2) no food should be left or eaten in the room; (3) hands, pens, pencils, and so on should be kept out of the mouth; and (4) hands should be washed at the end of the individual activity sessions.) Give each group a petri dish with nutrient agar (called a "culture plate" from now on). Have the children draw lines on the bottom of the plate to divide the plate into as many sections as there are children in the group, plus one extra section. This section serves as a control. (The lines can be drawn with a special pencil used for marking glass or china.)

Objective

The learner will define the term "control."

A control serves as a base line or normal condition which exists before the investigation begins. In a scientific investigation, a control provides the evidence which establishes the fact that the result of the treatment does not come from any place or any condition except from that already imposed. Only the factor being examined is omitted in a control. In the present investigation the empty section

of the petri dish provides the evidence that the colonies do not come from the agar or the petri dish. In the control section the agar should remain free from any colonies.

Within each group in the class, the hands of some of the pupils should meet the children's criteria of "clean," and the hands of some should meet their criteria of "dirty."

Now have each section of the plate imprinted by each of the children, with one finger placed directly on the surface of the agar. Label each section as having come from a clean or from a dirty finger.

When all the fingerprinting has been completed, invert the plates and place them in the incubator for 24 hours. You invert the plate (turn it upside down) to ensure that the surface of the plate remains free of liquid. If the plate is not inverted, moisture from the condensation of the agar will collect on the surface of the agar, and the colonies will not remain separate and distinct.

The temperature in the incubator should be stabilized at 35° C. (Explaining the relationship of the different measures of temperatures is appropriate at this point. The formulas for converting to centigrade and Fahrenheit appear in the definitions and explanations of technical terms in Section III. Since most of the professional literature uses the centigrade system – and it is assumed that some of the pupils in the sixth grade may wish to explore several professional books – the children should be able to convert to either scale.) If you do not have a commercially manufactured incubator, see the *Sourcebook for Elementary Science,* pages 13 and 14, for directions on building one.[1]

After 24 hours have passed, ask the children to examine the plates. In each plate there should be small, pin-like colonies of whitish material on the surface of the agar where the fingerprint was made. There may be many or few colonies. The plates should be examined daily for at least a week and observations made of any changes that have occurred.

Objectives

1. *The pupil will count, observe, contrast, and compare the different kinds of growth he sees on all the culture plates.*
2. *The pupil will evaluate the original criteria of "clean" and "dirty" in terms of the evidence available from the present investigation.*

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[1]Elizabeth B. Hone, Alexander Joseph, and Edward Victor. *Teaching Elementary Science: A Sourcebook for Elementary Science.* Under the general editorship of Paul F. Brandwein. New York: Harcourt, Brace & World, Inc., 1962, pp. 13-14.

3. The pupil will synthesize new criteria of "clean" and "dirty" in terms of the presence or absence of microorganisms.

In the body of the pupils' section of this guide, considerable stress is laid on the establishment of a system of recording observations. From the teacher's point of view, stress should also be laid on the need for the system to be kept very flexible. A system of recording is valuable only to the recorder. Nevertheless, the following criteria should be considered by both teacher and pupil in evaluating the system of recording data from observations, investigations, and experiments:

- Is the system valuable and workable for the particular child?
- Could that child, or someone else in the class, replicate his observations or his work on the basis of the information he has recorded?
- Is the child using a systematized approach to observation, to classification, and to the communication of his results?
- Is the child using terms that have a common meaning for all the members of his class?

Emphasis should be placed upon honest reporting of results regardless of whether they appear to be different from those of other investigators. In the field of science, the entire process of investigation involves an orderly exploration of the material universe, with the resulting knowledge being supported by evidence that can be validated by other scientists. Therefore, adequate time must be devoted to the development of a system and a style that are adequate to meet the criteria of recording. Extensions in terms of details should be encouraged, such as charts and drawings. Although each individual should be encouraged to develop his own system, the entire group and the entire class should be able to share and exchange individual systems and procedures.

An ongoing system of sharing information from each group or each individual engaged in special investigations must be established. The whole class should constantly evaluate (1) the findings of the groups or individuals; (2) the direction of new investigations; and (3) the clarity of the communication of information.

Many things are taught effectively through the sharing of negative results. It is quite probable that a number of the investigations will not succeed because of errors in procedure; for example, growth may occur on controls when they should remain clear. Experimentational failures should not cause the teacher or the pupils to be discouraged or unduly concerned. The possible causes of the failures should be

followed up; remedial suggestions should be developed by the children; and guidance should be given by the teacher. An error in the control will serve to point out beneficially (1) the importance of control in scientific investigation; and (2) the need for redoing the work.

Phase Four: Classification of Microorganisms

The learners have been led into a situation that necessitates observation. Not only have they used their eyes, but also they have had equipment to help them – in this case a hand lens.

Objective

In describing what he sees, the pupil will use terms that will be understandable to all who have been looking at the plates.

This objective begins the first synthesis of previous knowledge and the integration of the new knowledge that the children are pursuing. They should be aware that they are generating a vocabulary of terms which can be used to describe colonies that appear on the solid surface of a culture plate having a specific kind of nutrient. For gifted children who have above-average command of vocabulary, this task should be challenging but solvable. (A technical vocabulary describing the surfaces of colonies is included in Section III under "Classification According to Morphology.")

It should be stressed again that, while they may be helpful to the children, these terms are not sacred. It is true that professional bacteriologists or microbiologists use such terms to describe colonies and liquid growth, but other words may be more useful and meaningful to the children. However, it is important that the class as a whole establish the necessity of using terms that have the same meaning for the entire group involved and that the pupils work together to define the terms they intend to use. The terms chosen should be meaningful and should be accompanied by illustrations so that everyone understands the same meaning of each term and interprets it in the same way.

Objectives

1. *The learner will grow a culture in a liquid medium.*
2. *The learner will develop a list of definitions of terms to describe the appearance of the growth in the liquid medium.*

A second kind of nutrient medium can now be introduced – liquid broth contained in a culture tube. (See the technical section for preparation and sterilization procedures.) Ask the

question: Can organisms grow in liquid as well as on solid surfaces? (Answer: Yes, they can.)

Using a sterile swab, such as a Q-tip, the children can now plan investigations that involve rubbing the surface of some object with the sterile swab and then inserting the swab into the liquid broth. The swab is swished around thoroughly, and the excess liquid is pressed out of the cotton against the side of the culture tube. The cap is replaced, and the tube is incubated for 24 hours. The resulting growth is called a "mixed culture" because there will be several different kinds of organisms growing in the broth.

The pupils' section of this guide (Section II) contains information on the S-tube investigation, which is considered a classic type of bacteriological investigation. Historically, this method established that organisms do not originate through spontaneous generation but, in fact, come from the air. (See the technical section for detailed instructions on this procedure.) In the tubes that are directly exposed to the air and that allow the organisms in the air to fall into the broth, organismic growth occurs. In the tubes that are equipped with an S-shaped piece of plastic tubing on top, the air is forced to enter each culture tube in such a manner that no organisms are allowed to drop into the broth, and no organismic growth occurs.

Objective

The learner will perform pure culturing techniques.

From the mixed culture tube, the isolation of single colonies is carried out by using the "pure culture" techniques described in the technical section. The inoculating needle (or loop, as it is sometimes called) has a random sample of all of the organisms of the culture. By dragging it across the surface of a culture plate, the single organisms are dropped off, a few at a time. The last part of the plate to be streaked will contain colonies of all the organisms sufficiently separated that they can be picked up with the sterile inoculating needle and put into a sterile tube of fresh liquid broth. Now the new tube of broth contains only one kind of organism and is called a "pure culture." A new tube of broth should be inoculated with each different type of surface colony that can be discriminated as different. Each of these "pure culture" broth tubes should be kept fresh and active by transferring a single loopful of the culture to a new tube of nutrient broth each week. Cultures that have been allowed to grow in the incubator are called "24-hour cultures," as opposed, for example, to 48-hour cultures. Cultures that are not transferred weekly will become old and will eventually die out.

A small group of pupils can rotate the responsibility of maintaining fresh cultures on a weekly basis. Commercially prepared cultures containing known microorganisms are available. Once the culture has been started, there is no need to buy more of these, since they are quite expensive. Certain commercial cultures that produce different-colored colonies are interesting and are fun to use; they can be maintained over long periods of time if they are transferred carefully. (Refer to the back of this guide for sources of commercial cultures.)

The children should again observe the results and should develop the terms needed for their descriptions of the tests and the test results. The children's comments and reports should show, by now, an ability to classify successfully many of the microorganisms they have been examining.

Phase Five: Journeys into the Unknown

In this phase of the unit, the children should be ready to engage in more advanced experiments and to take on a greater variety of projects. Encourage them in their efforts. Motivate them to make known what appears to be unknown. Provide guidance whenever it is needed.

Objectives

1. The pupils will prepare stock nutrient media for inoculation.

2. The pupils will prepare liquid media for isolating organisms.

The technical procedures in Section III of this guide include precise directions for the preparation and sterilization of the media with which these objectives are concerned. The first major group of investigators should now be broken off from the class and given the assignment of preparing the media for the rest of the class. This should be a rotating assignment until everyone has mastered the techniques of "pouring plates" and of making and sterilizing various media.

Objective

The learner will prepare agar from raw seaweed.

A second group of learners who would be interested in trying to extract agar directly from raw seaweed might be organized. Since the pressure cooker for the sterilization of media is available in the room, the entire process can be carried out there. Before the children begin the actual process, encourage them to seek from encyclopedias the information they need on how to extract the agar. Sufficient information of this type is available to give them the clues that are

necessary for beginning the extraction process, but the information may not be so clear as to provide a specific recipe. Recommend to the children that they make several different kinds of attempts. Certainly the pupils should take careful notes for use when they report back to the whole class on the results of the project. This activity is optional. It may not be possible for children who live away from the seacoast – in areas where seaweed in not available – to engage in such an experiment. Some specific information on this kind of project is contained in the pupils' section of this guide under "Lesson One: General Information."

An extension of this activity can be applied by all interested pupils through the use of commercially prepared agar-agar. This is agar to which no nutrient has been added. By itself, it is inert; it does not support growth of organisms, just as the extract of seaweed prepared by the children will not support growth.

A suggested spin-off of some of the members of the class may be appropriate at this point: Interested pupils might wish to investigate certain kinds of nutrients that can be added to agar-agar so that this medium will be able to support bacterial growth. The criteria which these pupils should elicit for themselves regarding a particular nutrient are the following: "Will it support growth?" "How much growth will it support?" Several kinds of sugars, such as dextrose, levulose, and glucose, should be available for the children to manipulate. The pupils may want to experiment with many different kinds of foods. If this is the case, tell the children that it is very important to make careful notes as they proceed. Explain that these notes can be used for reports to the class, interaction with other members of the class, and the development of guidelines. Some of the learners may wish to spend all of their time on this part of the unit.

Objective

The learner will observe and classify bacterial colonies obtained from samples of air, water, and soil.

A small group of pupils may desire to continue the study of the morphology of bacterial colonies and to culture various objects or various samples of air, water, or soil with the intention of identifying as many different kinds of colonies as they can obtain. The changes that occur as the colonies age offer another avenue of exploration for those who might find this kind of investigation appealing.

Objective

The learner will make research reports about persons who have added to the knowledge of microbiology, and he will identify their contributions.

After an initial introduction to the materials, equipment, and techniques of microbiology, the children should be led to recognize that they are standing on the shoulders of others who have gone before them. To this end, another special group should be broken off from the class to learn about certain great men of science and medicine and how their contributions have affected the field of bacteriology. Such pioneers as Anton van Leeuwenhoek, Louis Pasteur, Robert Koch, Hans Christian Gram, and Joseph Lister should be studied, as well as some of the modern pathfinders – Jonas Salk, Albert Sabin, Alexander Fleming, Howard Florey, Selman Waksman, and others. The objective of the research might be to isolate the concepts, discoveries, and achievements that have made significant contributions to society and to its knowledge of microbes or bacteria. This activity, also, should use a rotating plan and should require the researchers to share their findings with the rest of the class.

Objectives

1. *The learner will inoculate a tube of liquid broth with a sterile swab that has been rubbed on the surface of some object or person.*
2. *The learner will transfer a loopful of this liquid broth to the surface of a nutrient-agar culture plate; he will use the streaking technique necessary to isolate single bacterial colonies.*

Those children who are not assigned to other areas of research or investigation can now begin the use of their procedural tools to investigate scientifically their own environment. The choice of what they would want to culture should be their own. For technical details on how to inoculate a tube of broth, see "Procedure Three" in Section III of this guide. For streaking techniques in the transfer of the broth culture to a solid medium, see "Procedure Four" in Section III.

Objectives

1. *The learner will design experiments on his own to obtain cultures whose colonies can be studied.*

2. The learner will investigate and validate advertising claims regarding germicidal properties of some products.

The entire class is now working in groups that have specific objectives. The objectives must be made known to the class, and the findings of each group must be relayed to the other pupils at regularly established intervals. The last group formed should have much leeway in the choice of what its members wish to explore. If the children need a certain amount of direction, specific suggestions which might be made include the following:

1. What would happen if the cover is taken off a petri dish and the surface left exposed for ten minutes? Would all parts of the room have the same kind of microorganisms?
2. Suppose a moldy orange or lemon were dropped in one corner of a room and several petri dishes were allowed to stand in various locations in that room. Would mold develop on them? How does mold get from one place to another?
3. What kinds of bacteria can be found on the skin, teeth, or hair of individuals? Can microorganisms be isolated from powder puffs or lipsticks? How about silverware? –feet of insects? –leaves of plants? –chicken broth?
4. What kinds of organisms might you find in tap water?
5. What is the cause of the black slime that forms on the edge of lettuce which is kept in the refrigerator for a period of time?
6. What makes a potato rot?
7. Is the advertising that reports the superiority of certain toothpastes and soaps as bacteriocidal agents really true?
8. What kinds of bacteria could be isolated from a bit of soil?

Within this class of gifted pupils, areas of interest should be identified and explored. Divergent thinking in the methods of obtaining specimens for culturing should be constantly encouraged. It is with respect to freedom of thought and action that the study of microorganisms has the most power for gifted children. They should not be forced to follow a prescribed format. Rather, they should be encouraged to work alone now as much as they wish, even though they may be functioning as part of a group.

The pupils should understand clearly, of course, that it is their responsibility to keep accurate notes and to record significant observations for the purpose of making progress reports both to their own small groups and to the entire class. At this point, all the work is being completed by using the morphology of the bacterial colonies as the basis of discrimination.

Phase Six:
"Project Micro-world" – Making the Invisible Visible

The last step to be introduced – in general ways to the class as a whole and in specific ways to the groups working on individual investigations – is the use of the microscope.

Objective

The learner will use a microscope to observe individual bacteria.

While the subject of microbiology can be studied without a microscope, certainly the use of this instrument would make the subject much more meaningful. The microscope is a critical piece of equipment which (1) makes it possible for the learner to see and study individual microorganisms; and (2) aids the learner in the classification and nomenclature of microorganisms.

Here again, it might be profitable for a few children to engage in special research – this time to study the literature on the microscope and how it operates and to report back to the entire class. (See the reading list for pupils toward the back of this guide, under "Resources and References.") This group of researchers can then serve as experts on the use of the microscope for the benefit of the group that will be applying the staining techniques.

What is important to the learners at this stage is the use of a microscope that has the power of bringing individual bacteria into the range of visibility. Such a microscope requires an oil-immersion lens. Since this type of instrument will probably have to be borrowed, it is essential that the children learn the proper use and care of microscopes. Those with oil-immersion lenses are available in most communities.

Objective

The learner will stain individual microorganisms.

Because most bacteria are tiny and translucent, they are difficult to study, even under a microscope, unless they are stained. The techniques for preparing slides for different kinds of stains and the techniques for staining the slides are described in Section III under Procedure Nine.

Ideally, the groups of pupils who will be engaged in staining and microscopy should be organized as soon as bacterial colonies have been obtained on the culture plates. The staining group can investigate these colonies in terms of individual organisms and their staining properties, and the microscopy group can gather important information through accurate observation of surface colonies. These

two groups will work closely together and will assist each other. The interaction and interdependence of the two groups should demonstrate effectively the concept of the interdependence of man.

The specific techniques for staining a slide after a single colony has been isolated on a culture plate are presented in the technical section under Procedure Nine. Questions such as "Why do we have to pass the slide containing the organisms through a flame?" may arise. Passing the slide through a flame makes the organisms adhere to the slide so that they will not be washed off by the stains and by water. This technique is like frying an egg in a non-Teflon frying pan – the protein solidifies and adheres to the surface of the pan.

The children who are now on the microscopy team will undoubtedly wish to investigate the staining properties of colonies and individual organisms by using the techniques suggested in the technical section.

The most useful stain is the Gram stain (after Hans Christian Gram), which divides the class of microorganisms known as bacteria into two major groups called "Gram-positive" and "Gram-negative." Those organisms which retain the purple stain after having been decolorized and counterstained with pink stain are the Gram-positive organisms. Those organisms which readily give up the purple dye and, therefore, appear bright pink in color after having been counterstained are designated as Gram-negative. The staining properties of the organisms are not related to the morphology of the individual organisms.

Bacteria are also divided into three distinct groups according to their morphology:

1. *Cocci.* These are round in shape; they often appear singly, sometimes in a cluster, and occasionally in chains.
2. *Bacilli.* These are rod-shaped or cylindrical; some have ends that are very flat, while others have ends that are so rounded that they appear almost spherical. There is much variation as to size and as to ratio of length to width, and the shape of the individual organism tends to change with the age of the culture.
3. *Spirilla.* These are spiral-shaped and look very much like corkscrews. They are not as widely found as the two other kinds.

For those pupils who are studying the staining properties of microorganisms, pure cultures are the most convenient sources of bacterial supply. One precaution, however, should be mentioned here as an aid to the pupils in maintaining the cultures. The inoculating loop that is used to obtain the loopful of culture medium must

always be sterilized by heating it in an open flame and making it red-hot before it is inserted into the culture tube. After the culture drop has been transferred to a slide, the inoculating needle must again be flame-sterilized. At no time should it ever be set down flat on a table or desk. If the pure culture happens to become contaminated, it can be restored by streaking another loopful of the culture on a culture plate and picking up a single colony of the organisms again; this colony can then be transferred to a new tube of liquid broth, and another pure culture is available.

Phase Seven: Disposal and Sanitation Techniques

The final phase of this unit involves the disposal of the used culture materials as well as a general cleanup of the room.

Objective

The learner will safely dispose of used materials and cleanse the working surfaces in the room.

Plastic sacks, such as those supplied by grocery stores for vegetables, make excellent disposal containers. The plastic petri dishes and swabs should be discarded after use; they should be closed up and placed inside the bags. Then the bags should be sealed tight with a plastic wire and disposed of in the manner prescribed by school sanitation regulations.

Because the petri dishes are plastic, the usual methods of sterilization by heat and pressure cannot be used. Moreover, children who are involved in this type of study should not be expected to clean this kind of equipment. Since the cost of the dish is nominal, the petri dishes should be treated as expendable. Incineration is considered the best method of disposal.

As pointed out earlier in this section (see the list of rules in Phase Three), a disinfectant solution should be used at the end of each working period to wipe down the desks and tables.

Summary

You, the teacher, can readily perceive that this unit allows for (1) a great deal of individual study and activity; and (2) considerable flexibility as to what is to be studied. You realize, at the same time, that interaction and interdependence on the part of the entire class is germane as well as important to the progress of the learners. You recognize, moreover, that the class does not move as a single unit, except at the beginning, but that spin-offs occur in many areas of research and activity. These spin-offs provide opportunity for

in-depth study according to the individual interests of the children. You can reasonably assume that eventually all the pupils will move through all the techniques to a final development of investigations and experiments designed by the pupils themselves.

The depth and the latitude of the learning opportunities suggested here depend upon the abilities and interests of the learners. In all probability, few of the children will be interested in extending their learnings in this field beyond the subject matter presented in this guide. However, if some of the children wish to probe the reactions of pure cultures of organisms in the presence of different sugars in order to determine those which produce acid and gas, or to measure the change in *pH* of a culture medium[2], or to investigate the action of microorganisms on proteins such as milk, you can refer them to more sophisticated books on bacteriology and encourage them to continue their research. By this time they will have acquired all the basic techniques with which to carry on investigations of this nature. You might also discuss with them the ideas presented in the Addendum to this guide – ideas that have to do with disease-producing organisms. The content suggested by the Addendum could be used for a second unit on microbiology.

In terms of the processes of scientific inquiry and investigation, the present guide provides the children with opportunities to utilize all of these processes, which include observing, classifying, measuring, communicating, hypothesizing, testing, analyzing, drawing conclusions, and evaluating results and conclusions. As long as the subgroups of learners continue to meet with the rest of the class to present the facts they have found, to share their ideas, and to report the results of their progress, the concept of the interdependence of scientists in exploring environments will be made clear. The children will be "sciencing" as scientists do – with few clear-cut formulas to follow, with no rigid sequence of steps to comply with for the sake of compliance or conformity. But they will use the processes of science and the tools of microbiology in accordance with what they think is necessary to know and understand the world of microbes.

It is rather hoped that this area of learning is new to you, the teacher, as well as to the children you teach. If this is the case, the subject of microbiology can be explored both by you and by the gifted learners in your charge. Much can be learned, even if everyone has to wonder a little. Yes, mistakes will be made, but the freedom to make them and to learn from them is a right that all children and all adults should have; otherwise, education is impossible.

[2]The letter combination of *pH* is the chemical symbol for the logarithm of the reciprocal of hydrogen ion concentration in gram atoms per liter.

Section II

SUGGESTED LEARNING ACTIVITIES

This section, addressed to the pupils, consists of four sample lessons on microbiology. The learning activities proposed in these lessons for gifted children in grades four through six should not be hampered by rigid schedules. Rather, the activities should be part of a continuous flow of opportunity, of communication, of exchange and sharing, and of movement and development according to the individual interests and abilities of all the members of the class.

The scientific investigations, experiments, research, and projects suggested here (1) are to be utilized in conjunction with the instructional approaches described in Section I of this guide; and (2) can be aided and enriched by the technical information presented in Section III as well as by the reading materials cited under "Resources and References." Although many of the activities are suitable for groups, subgroups, and individuals, the entire class needs to benefit from the pupils' experiences. Frequent interchange of ideas and findings is most important to the progress of the children, both individually and collectively.

Because the four lessons in this section have been developed expressly for the learners themselves, nearly all of the content has been written in the second person.

Lesson One: General Information

Is there anyone who doesn't remember having his mother say, "Go wash your hands – dinner's ready" or "Don't touch that dog – he's dirty" or "Don't eat that candy – it was on the floor"? Have you ever looked at your hands and thought to yourself that they didn't really look very dirty? In fact, they looked quite clean; you just washed them ten minutes ago. Have any of you ever asked an adult – perhaps your mother or father or an aunt or an older brother– what that person meant by "dirty hands"? What do you mean by the word "dirty"? What do you mean by the word "clean"? Can you think of a list of conditions which you might establish as

criteria for dirtiness or cleanliness? Is your list the same as your next-door neighbor's? Do you have a common understanding of such terms as "dirty" and "clean"?

Perhaps you have had someone tell you that things have germs on them or bugs on them. Do you understand any better when people say that there are germs on your hands? that there are germs on a piece of candy? that there are germs on a dog? Have you ever seen a germ? How do you know that germs exist?

Most of you have a good idea about the general direction that you are going to take in this study of microbiology. Most of you understand what is meant by the term *biology*. Now, the term *micro* means *small.* If you put the two words together, you have a term that means the study of tiny living things in the world around you.

This kind of study, however, has not been with us very long. As late as the seventeenth century, people did not know anything about the invisible world of microbes.

After the microscope was invented and used, it was discovered that these tiny organisms existed in the water and in decaying matter. But how they came into existence was a question which the scientists could not answer for quite a while. Where did these organisms come from? Where did they go? Did they have any parents? Were they really alive? – and, if they were alive, what did they eat? Did they eat in the manner of living things with which most people are familiar? How did they multiply?

Slowly over the years, the answers to questions like these have been found, and much information has come down to us. We could go back and repeat many of the early investigations, but perhaps we should do what most scientists do when they come into contact with a new problem. They go to the library. They obtain as much information and knowledge as they possibly can from pioneers and investigators who went before them. So it is not necessary for us to go back and rediscover everything that has already been discovered. We can depend upon the discoveries which others have made and the trailblazing which others have done in order to acquire more knowledge for ourselves.

Under "Resources and References" at the back of this guide, you will find a list of recommended books that can add to your general knowledge about microbiology, particularly about bacteria, molds, and yeast. These books will give you some useful, interesting information about the characteristics of bacteria, molds, and yeast; about their environments and the places where they can be found; and about the conditions under which they grow. The books are relatively easy to read; you are asked to read as many of them as you

can to provide yourself with a good background of knowledge. It might be wise, also, for you to check the encyclopedias and reference books in your library under such headings as Agar, Algae, Bacteria, Fungi, Germs, Media, Microbes, Microbiology, Molds, Nutrient, Nutriment, Yeast, and any other headings which you feel would lead you to material that would add to your store of information.

While you are doing this research, keep in mind that some pertinent questions might occur to you; jot them down. Take notes as you study. Begin to work as a scientist does. Remember, the notes you record are for *you*. They are not to meet the needs of others. Make your notes as detailed as you need them to be so that you will remember the major ideas. Organize them in a way that makes the information understandable to you and readily available to you at any time.

Lesson Two: Basic Investigations

At the beginning of the last chapter, you were asked to define "clean" and "dirty." With the knowledge that you have gained from your library research, have you decided upon any additional criteria that can be used for these terms? Are these adequate words, really, to work with in the field of microbiology? You might divide into small groups now; within each group observe your hands. It is hoped that in each group some will have hands that meet your criteria of cleanliness and some will have hands that meet your criteria of dirtiness.

Investigation 1

Now you can try the first investigation and see what kinds of results you obtain. The following materials should be used:

- Petri dishes containing nutrient media
- A marking crayon
- An incubator

Your teacher will give you a round, plastic dish or plate that has a cover on it. On the bottom of this dish you will see a layer of what appears to be a gelatin-like material. Don't take the lid off the dish at any time while you are examining it or marking it. Now take a red crayon or a special pencil used for marking glass or china. Turn the plate over. With your marker divide the backside of the plate into as many sections as there are pupils in your group, plus one additional section. These sections will look like pieces of a pie. Label them 1, 2, 3, 4, and so on. Then label one section "control." Turn your plate right side up.

On this page you will see a picture of one of these plates, which is called a petri dish. Can you manage to lift the lid just a bit so that the surface of the medium in the plate is not totally exposed? Be very careful. Now, each of you should take one finger and press it into one section of the agar. Press firmly so that your fingerprint shows. If space is available, press another finger on another part of your section of the plate. Be sure to label the back of your plate with an identification number so that you will remember which section has your fingerprints on it. Remember, you must never take the lid off the dish completely.

One section, the control, has nothing on it. Now replace the lid on your plate; turn the plate upside down and put it in the incubator for 24 hours. At this time you should be asking such questions as the following: "What is a control?" "At what temperature should I set the incubator?" "Why must I turn the plate upside down?" "Why can't I take the lid off the plate?"

PETRI DISH

Closed *Opened*

You may now add to your original notes the additional information you have found about your investigation. Remember, the format of your note recording is strictly up to you. You should, however, include enough information on what you did so that someone else could repeat exactly what you accomplished and obtain essentially the same results. This process is what is called scientific verification. Be sure that you include the date of your experiment, what you did, the temperature at which you incubated your plate, and the results.

What do you think is going to happen in the section labeled "control"? Before your plate comes out of the incubator, discuss that question with your group. Try to figure out what a control does. Now check with the rest of the class to determine whether each group has reached the same conclusions as yours.

You were making some basic assumptions when you started. Do you know what you were assuming about that plate before you began the culture process? What is meant by the term "sterile"?

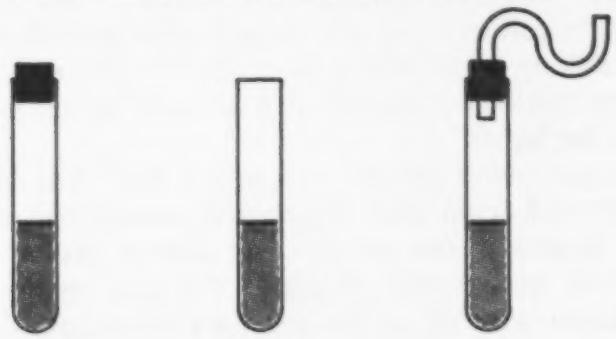
Investigation 2

While you are waiting for this first investigation to come out of the incubation stage, begin your second investigation. The following materials should be used:

- Three sealed tubes containing liquid broth culture
- One S-shaped tube
- One tube rack

What would happen if you took two tubes of liquid culture and exposed one to the open air, simply by taking the lid off the top of it? Leave the other tube capped or covered. Now, set up a third tube; this one is open, but it has an S-shaped piece of plastic tubing for the cap. With this arrangement, the third tube is open to the air, but the air must follow the pattern of an S before it gets into the tube itself. Your teacher will provide a set of tubes of broth for each of your groups. Again, have you hypothesized what will happen? Have you described exactly what the setup is for this experiment? Which tube is your control?

TUBES CONTAINING BROTH CULTURE



Sealed tube *Open tube* *S-tube*

Decide in which part of the room you wish to leave your tubes. A portion of the room which is dusty or which encounters a great deal of foot traffic is the most desirable place. Why? Make sure that your tubes will not get knocked over. Why? Remember: one tube is exposed to the air; one is sealed; and one is exposed to the air but is fitted with an S-shaped piece of tubing. Set all three in a large cup or in a test-tube rack and observe them each day, or every two or three days, and then make a final observation after two weeks have passed.

What were your results? What happened? Why? Do you think that microorganisms spontaneously spring into being, like a rabbit out of a hat? Where do they come from? What kind of evidence can you

cite for your answers? Again, what assumptions were you making about your materials when you started to investigate? What happened after 24 hours? What happened after two weeks? Is your system of recording what you are doing an adequate one? Do you need some help from your teacher in organizing your notes? Have you checked with your group to compare ways of writing things down? Has your class decided on any kind of format that might be helpful to everyone?

A Return to Investigation 1

Return to your first investigation. Obtain hand lenses from your teacher.

You have two investigations under way. Now observe your first investigation again. What has happened after 24 hours? Perhaps nothing; if this is the case, wait another 24 hours.

Take a hand lens and look very carefully at what you see. Can you adequately describe what you find? Each one of those little dots on your section represents a colony that is composed of thousands of single organisms. Look carefully. Like yourself, they have special characteristics. Can you develop a vocabulary that will adequately describe the colony's general appearance? Can you describe the surface of the colony? Can you describe the growth around its edges? Does the colony have color? Does it have an odor? Does it look as though you could see through it if it were big enough and if it were held up to the light?

Did you get many colonies, or just a few? Are they all the same? Does the growth from your finger look exactly like the growths from the other fingers in the group? – or does it appear to be different? Are you able to describe all of the colonies on the plate? Can you compare them with all of the colonies belonging to another group? Are they all essentially the same? – similar? – different? What would you say about the terms "clean" and "dirty" now? Is there a significant distinction?

An Extention of Investigation 1

It will be interesting now to extend your first investigation. The following materials should be used:

- Petri dishes containing nutrient media
- A marking crayon
- An incubator
- Sterile swabs
- Different kinds of soap

This time your group should be divided into two teams. The members of one team should wash their hands very, very thoroughly with different kinds of soap. Make careful notes as to who washed with what kind of soap. You hear many commercials on radio and television about the germicidal power of certain soaps. It might be interesting to check whether these soaps are, in fact, what they are represented to be. The members of the other team will not wash their hands. Instead of putting your finger directly on the agar, take a sterile swab (one for each pupil) and rub it across your hands and on the tips of your fingers. Then gently stroke the surface of the agar with these swabs. Again, mark divisions on your plate and identify each division. (Remember, arranging and setting up your investigations *first* saves you much confusion.) Follow the same procedure as before. Invert your plate and put it in the incubator for 24 hours.

Now what do you find? Is there a difference between the growths produced by the hands that were clean and the hands that were dirty? Is one soap more effective as a cleaning agent than another? (Bear in mind that this is a *real experiment,* because no one will really know the answer until your work is completed.)

In order to control your experiment, what did you do to one section of your plate? Did you have each pupil wash for the same amount of time? Did each pupil who was washing rinse carefully and thoroughly so that all the pupils' hands were given essentially the same treatment? If not, repeat your investigation.

What should you do if your control section develops growth? You know by now that unless your control remains sterile, you can draw no valid conclusions from your experiment. It must be done again and again, until your control remains without growth.

Lesson Three: Proper Use of the Tools

In this lesson you will learn the proper use of scientific tools in your study of microbiology. Groups and subgroups of pupils in your class will be engaged in some interesting tasks and experiments in which these tools and appropriate ways of using them will be given close attention. As in the other lessons in this unit, it is important for you to share your findings with the rest of the class.

Preparation of Media (Group 1)

Some of you will now be asked to prepare certain kinds of media, over a short period of time, for the entire class. In addition to the materials already on hand, a weighing device known as a "balance" will be needed.

In Section III under "Technical Procedures," you will find specific instructions for making tubes of liquid broth, as well as for pouring plates of nutrient agar. Your teacher will have all of the necessary supplies, but you must put all of the materials together and learn how to pour plates so that they remain sterile and ready for use by the class. If you have a balance in your room, you can learn to measure the proper weight of the nutrient agar in grams and to measure the volume of the distilled water in cubic centimeters. These are the dry and liquid measures, respectively, which are most commonly used in science. Find the answers to the following questions: One quart of water is about how many cubic centimeters? One gram is how many ounces?

You will also note that the instructions in the technical section are given in terms of centigrade temperature. Can you convert *centigrade* to *Fahrenheit?* What are these terms? What do they mean? Do you know how to make the change from one scale to the other? It might be important for you to know how to make this conversion, because temperatures are very often indicated on the Fahrenheit scale, especially those with which we are all familiar; for example, the temperature of the air as to degree of heat or cold, or the temperature of the body as to normal level of warmth.

In science, however, the centigrade scale is widely used. Do you understand the relationship between the two scales? For instance, 37° centigrade is the equivalent of 98.6° Fahrenheit. With these temperature figures at your disposal, do you know how to make this conversion? If you do not, whom would you ask or what sources would you check to find out? Let us assume that you have learned the right formulas and the correct methods of computation. Can you now convert 100° centigrade to its equivalent on the Fahrenheit scale? How about 80° Fahrenheit to centigrade? What temperature is your incubator thermometer reading at this moment? What temperature is your room thermometer registering?

Extracting agar from seaweed (Subgroup 1). In this activity the following materials should be used:

- Seaweed
- A pressure cooker
- Bottles with screw caps
- Petri dishes
- A funnel

Those of you who are making media for the class have probably learned by now that the kind of material you are working with is called "agar." Where does it come from? What does your encyclo-

pedia have to say about agar? If you live on the California seacoast, do you have seaweed on your beaches? If you do, some of you might find it interesting to bring in a quantity of that seaweed and try to extract agar from it.

How much seaweed should you have initially to obtain enough agar to fill a small bottle? If you have already completed the extraction process, what methods did you use? Did you chop the seaweed, cut it, boil it, pressure-cook it, dry it, or grind it? What did you do? The processing of agar is a young industry, even at the present time. The methods of extracting this substance commercially are very similar to the one you and your classmates are using. If you are still working on this project or if you are trying it again, perhaps you can find a way to extract a more pure type of agar than has been developed up to now. Be sure to keep careful records of what you are doing. Have you encountered any kind of seaweed that does not render agar?

Conducting an alternative investigation (Subgroup 1). In this activity the following materials should be used:

- Agar-agar powder
- Distilled water
- A pressure cooker
- A balance for weighing and measuring
- Bottles with screw caps
- Petri dishes
- A funnel

For those of you who do not live near the seacoast and cannot extract agar from seaweed, an alternative investigation is suggested. Obtain some agar-agar powder from your teacher. Measure the prescribed amount of powder and add the proper amount of distilled water. Boil and bottle the liquid agar. Sterilize your product in the same manner that you sterilize the nutrient agar plates. Now pour some of the sterile liquid agar into several petri dishes and allow the agar to set for a while.

Discovering the need for nutrients (Subgroup 2). For those of you who have extracted agar from seaweed and have sterilized it in exactly the same way that was followed when nutrient agar plates were prepared for the class, there is a special kind of investigation which you might find interesting. Take some of your sterile agar extract, melt it, and pour it into a sterile petri dish. Repeat any of the investigations which you have made up to this point. What do

you find? What has taken place? Are your results different from those which you observed when you used the nutrient agar? What can you conclude about the nutrient value of agar extracted from seaweed?

For those of you who used the agar-agar powder instead of seaweed, your next step is much the same. Take your poured petri dishes and repeat any of the investigations you have made up to this point. What happens? Why? What must have been added to the agar you used before but is not present in this agar? Does the term *nutrient agar* have more meaning to you now?

Investigations with nutrients (Subgroup 3). If the idea of manipulating (changing) the nutrient part of a microorganism's environment appeals to you, it is possible for you to design many experiments in which you can use plain agar as an inert base and add food substances that can serve the purpose of nutrients. For instance, you can prepare a beef extract in the following manner:

Boil a piece of beef until all of it consists of very small pieces; then strain the pieces, take the liquid that is obtained, and add the liquid to your agar. The treated agar becomes one kind of nutrient base.

You might try the same process with chicken and with other kinds of foods that can be added to the agar base. Do you suppose that an extract of a carrot, when added to plain agar, might be sufficiently nutritious to promote the growth of microorganisms? It is quite possible that *that* kind of nutrient has never been tried before. Why don't you be the first to work with certain foods other than those suggested?

Staining Techniques (Group 2)

Another major group of learners can be exploring staining techniques according to the procedures set up in the technical part of this guide (Section III). These techniques are important in activities involving observation and classification.

Microbiological Classification (Group 3)

A third major group with which this lesson is involved can be using the services of the staining group and the pure-culturing group to learn more about the morphology of microorganisms and their colonies.

If you are working in Group 3, you will need to learn how to operate a microscope and how to record your findings in the use of that instrument.

If you discover a specific kind of organism that seems to be nourished by one kind of food and does not seem to be nourished by

other kinds, you may want to find out just what type of organism you are dealing with. You can even look up its name in *Bergey's Manual of Determinative Bacteriology.*[1] This useful book identifies the morphology and staining properties of individual microorganisms and the chemical reactions of these microorganisms to certain sugars, as well as other reactions to proteins and sugars.

Effects of Radiation on Specific Cultures (Group 4)

In addition to the group that might be interested in doing extensive work on nutrition, still another major group might want to check the effects of certain phenomena or processes, such as ultraviolet radiation, on specific cultures. In this activity the group would use petri dishes containing pure cultures of a single type of microorganism.

Consider the possibility of causing a plate containing colonies of organisms of a single type to be subjected to various levels of radiation, transplanting some of these colonies to broth cultures, inducing successive generations, and checking the morphology of both the colony and the organism. Are they the same? Is there any variation in size? Do the colonies continue to appear the same, or can you produce certain changes? For example, can you bring about a change in which a smooth-edged type of colony becomes a rough-appearing type?

Lesson Four: The Challenge of Experimentation

Now that you have built up a fund of information about microbiology, have carried out some basic investigations, and have learned the proper use of scientific tools, you are ready to do quite a few things "on your own." At this point you are more knowledgeable and more competent; best of all, you are more self-reliant.

Some of you may have some original ideas and plans and may want to put them to the test. Others may want to extend or enlarge upon several of the investigations that have been made thus far in this unit. Still others may wish to launch into well-known, important experiments with which the class has not as yet been involved. In any event, this can indeed be a time of challenge, excitement, and valuable learning.

There are many directions you can take. The following topical questions are suggested for your consideration (no doubt you will think of others):

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[1] David H. Bergey, *Bergey's Manual of Determinative Bacteriology* (Seventh edition). Edited by Robert S. Breed and Others. Society of American Bacteriologists. Baltimore, Md.: Williams & Wilkins Company, 1957.

1. What specific areas of microbiology would you like to investigate now that you have the basic tools and techniques?

2. What kind of environmental experiment would you like to design? – for example, a design that would allow you to introduce in the environment of microorganisms a change sufficient to alter, perhaps, the genetic pattern of the organisms.

3. Are you interested in finding out what happens to organisms that are dried for varying lengths of time? Do they always die?

4. What might happen if you changed the pH[2] of your media by adding acids or bases? Do you suppose that some cultures might give off gas as they grow? How could you determine or measure these factors?

5. Do molds have *colony appearances* in the same way that bacteria do? What kind of nutrients would you add to an agar base to grow molds luxuriantly? Where would you go for your cultures? Do you need some new terms to describe molds?

6. How would you study yeasts? What experiments would you like to set up? Would you feel that new or additional terminology is necessary to describe yeasts?

You have most of the tools you need to explore the world of microbiology. The only limiting factors are the extent of your curiosity and the patience you must develop to explore systematically and carefully.

Happy "sciencing"!

[2]The letter combination of *pH* is the chemical symbol for the logarithm of the reciprocal of hydrogen ion concentration in gram atoms per liter.

Section III

TECHNICAL TERMS AND PROCEDURES

This section contains detailed information of a technical nature. The explanations, descriptions, data, and procedures presented here are intended to assist both the teacher and the pupils as they proceed through this unit on microbiology. Within the steps suggested in sections I and II, frequent use can be made of Section III, which serves as a kind of backup or "information center" for the unit. Many details regarding terminology and procedures not found elsewhere in the guide may be found in this part.

Section III consists of three subsections: (1) "Definitions of Terms"; (2) "Aspects of Classification," involving morphology and liquid culturing; and (3) "Technical Procedures," comprising a sequence of ten procedures that relate directly to the subject matter of this guide.

Definitions of Terms

Agar – A gelatin-like material that is solid at incubator temperatures. It is used for culturing bacteria.

Bacteria – Microscopic one-celled (unicellular) organisms usually classified as plants. They lack green coloring (chlorophyll), and they divide by fission. Because they are tiny living things, they are rightly called microorganisms.

Bacillus (pl. *bacilli*) – A microorganism that is rod-shaped or rectangular.

Coccus (pl. *cocci*) – A microorganism that is spherical or round in shape.

Spirillum (pl. *spirilla*) – The "comma," or spiral-shaped microorganism.

Centigrade – The temperature scale used most often in the field of science. It has a range of 0 to 100 degrees. The formula for converting centigrade temperatures to Fahrenheit temperatures is the following: $F = 9/5\ C + 32$.

Colony – A group of bacteria that grow together in solid media. A single colony is composed of a mass of single organisms. After about 24 hours of growth, a colony is visible to the naked eye. It possesses certain distinctive, observable characteristics described in the classification portion of this section.

Culture – An active growth of microorganisms on or in any kind of medium, whether solid or liquid.

Mixed culture – A culture that consists of two or more species of organisms growing together.

Pure culture – A culture that consists of only one species of organism growing in or on a nutrient medium.

Fahrenheit – The commonly used scale of measuring temperatures. It has a range between 32 and 212 degrees. The formula for converting Fahrenheit to centigrade is the following: C = 5/9 (F–32).

Gram-negative – The term ascribed to a major group of bacteria which do not hold the primary purple of a Gram differential stain, but which give up the purple stain easily. Such bacteria then retain the pink secondary stain, called a counterstain. Gram-negative organisms appear pink under a microscope.

Gram-positive – The term ascribed to a major group of bacteria which hold the first crystal-violet stain in a Gram differential stain. The bacteria thus appear purple under a microscope.

Incubator – A container in which the temperature can be regulated and thermostatically controlled to maintain specific heat requirements for the growth of microorganisms in culture plates.

Inoculating needle – A thin wire with a loop at the end, used to transfer microorganisms.

Media – The liquid or solid substances in or on which microorganisms are grown. In the solid state, a medium is composed of agar plus various combinations of nutrients that supply carbon and protein for the metabolism of the microorganisms.

Microorganism – The term applied to the simplest forms of plant or animal life. Single microorganisms are invisible to the naked eye.

Morphology – The external appearance of a single microorganism or of a colony of microorganisms. (See the classification portion of this section.)

Mycology – A special study of fungi and molds: their morphology, their cultural requirements, and their effects on the environment.

Nutrient – A carbohydrate or protein added to agar to supply the source of energy that is needed for the growth of the microorganisms.

Petri dish – A small plastic dish or plate equipped with a lid that fits snugly over the top. It comes in a sterile state, and the lid should not be removed until the dish is ready to be used.

Streaking – The technique of moving a needle or a swab back and forth across a petri dish in order to deposit bacteria on the surface of the agar.

Yeasts– Single-celled organisms that are larger than bacteria and that reproduce by budding.

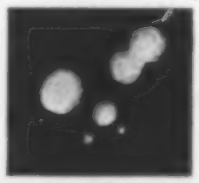
Aspects of Classification

The following information, accompanied by several simple illustrations, has to do with certain aspects of microbiological classification: (1) classification according to the morphology of bacterial colonies (form, shape, structure, general appearance); and (2) classification terminology commonly used to describe liquid cultures.

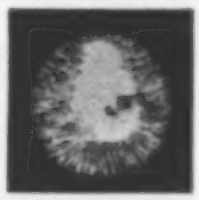
Classification According to Morphology

Form of the Colony

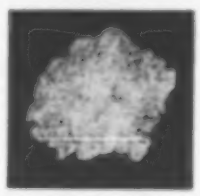
Circular: in the forms of round clusters, dots, or pinpoints



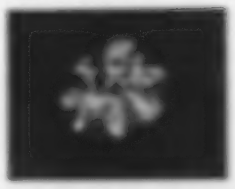
Filamentous: round, but with filaments or strings or hairlike projections coming from the center



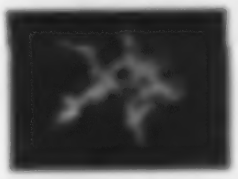
Irregular: having protrusions from the center



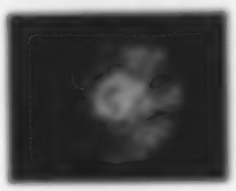
Curled or lobulated (having lobes)



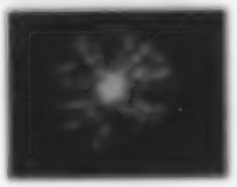
Amoeboid: having fingerlike projections that extend from the center



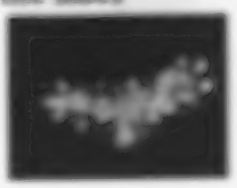
Mycelioid: appearing round, but with filaments extending evenly from the center



Rhizoid: appearing round, with many rootlike filaments branching from the center



Toruloid: appearing longitudinal rather than circular, and lobulated on the ends and the sides



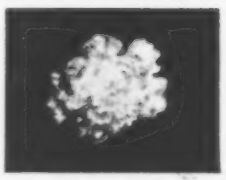
Appearance of the Colony

Glistening	Transparent
Dull	Opaque
Chalky	Translucent

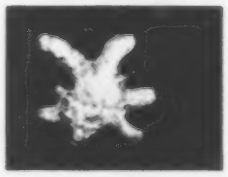
Edges of the Colony

Round and even

Having uneven lobulations



Amoeboid – showing fingerlike projections from the center



Lobulated – having irregular cuts

Surface of the Colony as Viewed from the Side

Flat

Raised, with a knot at the center

Thick

Thin

Convex

Concave

Hemispherical

Having a depressed center

Smooth

(Drawing can be made by teacher or pupil.)

Fuzzy
Rough
Bristly (showing spikes from the center)
Wrinkled
Ridged

} Drawings can be made by teacher or pupil.

Odor of the Colony

Absent or none
Slight
Strong
Resembling another odor (to be specified by the pupil or by the teacher)

Amount of Growth in a 24-hour-old Colony

Slight
Moderate
Abundant
None

Classification Terms That Are Used to Describe Liquid Cultures

Amount of Growth in a 24-hour Culture

Slight
Moderate
Abundant
None

Surface Growth

Turbid – indicating a kind of cloudy growth in general
Granular – having small pieces of growth in the liquid, especially at the bottom

Growth at or Near the Bottom of Liquid Media

None
Slight
Moderate
Persistent
Strong
Transient – showing clouds that appear to clear up in time, with solids dropping to the bottom

Odor

Same terms as those used for colonies grown in or on solid media

Technical Procedures

Concise information on materials, equipment, and methods in conducting microbiological experiments and investigations is presented in the ten sets of directions that follow. The procedures described here should be useful for reference and guidance as both the teacher and the pupils explore the content suggested in this unit.

Procedure One: Making Nutrient Agar

1. Measure 40 grams of nutrient agar powder on a balance. Place in a large flask, preferably one that has a capacity of 1,000 ml (milliliters).

2. Measure 1,000 ml of distilled water. Add to the powdered nutrient agar in the bottom of the flask.

3. Stir the mixture gently until it has partially dissolved.

4. Bring the mixture to a rolling gentle boil to ensure that all particles of the agar have been dissolved.

5. With the use of a funnel, deliver approximately 100 ml of the liquid agar into 150-ml prescription bottles. These bottles should have screw caps. (*Caution:* do not fill the bottles with more than 100 ml of the agar.) Each batch of 40 grams of agar should produce essentially 10 stock bottles of nutrient agar.

6. Place the bottles – with the screw caps attached but loose – in a home-style pressure cooker. Depending on its size, the pressure cooker will hold between six and eight of the bottles for each run. Add ¼ cup of water to the bottom of the pressure cooker. Be sure that the bottles are on some sort of platform inside the cooker. Bring the pressure up to 15 pounds; maintain that pressure for 20 minutes. At the end of 20 minutes, allow the pressure to come down slowly.

7. Allow the bottles of agar to cool. This supply of filled bottles represents a stock of sterile agar, which, when cool, will be hard or solid.

Procedure Two: Making Liquid Broth

1. Measure on a balance 8 grams of a powdered nutrient broth medium (a specially prepared beef extract or soy broth).

2. Add this to 1,000 cc (cubic centimeters) of distilled water. Mix.

3. Bring to a gentle boil to assure complete dissolution of the powder.

4. Place 10 cc of the liquid broth in test tubes with screw caps. Each tube should be filled with the medium to a capacity not exceeding ¾ of the tube.

5. Place the tubes upright in a pressure cooker and pressure them at 15 pounds for 15 minutes. Be sure that the caps are loose.

6. Cool the tubes and tighten the caps.

Procedure Three: Inoculating Liquid Broth

1. Liquid broth may be inoculated by the direct insertion of a swab that has rubbed the surface of the area to be cultured. Swish the swab in the medium and squeeze out the excess by pressing the swab against the side of the tube. Remove and discard the swab.

2. Liquid broth may be inoculated by using an inoculating needle to transfer a colony from a solid surface to the liquid broth. The following sequence should be used:

First, flame the needle in an alcohol lamp until it is red-hot.

When the needle is red-hot, insert it under the lid of a petri dish and touch a sterile area of the solid agar. This action cools the needle quickly.

Now pick up a single colony with the end of the needle, replace the lid of the petri dish, and transfer the solid single colony to a tube containing a liquid medium.

Insert the needle into the liquid medium and stir the liquid with the needle. Use the needle as a stirring rod.

Withdraw the needle and flame it again to red-hot. (It should be noted that the cotton stopper or the screw cap belonging to the tube of liquid broth must never be placed on the desk. Instead, the technique of holding either the cotton plug or the solid screw cap in the crook of the small finger should be mastered.)

All tubes, as well as all petri dishes, must, of course, be adequately identified before and during the procedure.

Procedure Four: Streaking a Plate

1. Rub a sterile swab across the surface to be cultured.

2. Then rub the inoculated swab gently on the surface of the agar. Care must be taken to ensure that physical contact is made between the surface of the agar and the swab containing the organisms to be cultured.

3. When sterile swabs are directly applied to the surface of the agar, it is the usual procedure to start at one edge of the dish and to proceed across the dish from side to side in close, continuous, sweeping motions. (See illustration.) It is important that there be no backtracking over the same area inoculated, inasmuch as the sweeping motion drops off organisms a few at a time and single colonies can thus be isolated and studied.

STREAKING THE SURFACE

4. The inoculated plates are then inverted and placed in the incubator for the specified period of time.

Procedure Five: Conducting an S-tube Experiment

1. Take three tubes of sterile nutrient broth. Stand them upright in a tube rack.

2. Tube #1: Label it "control"; do nothing to this tube.

3. Tube #2: Take the cap all the way off.

4. Tube #3: Bend a piece of plastic tubing into the shape of an S. (All you need is a 3- or 4-inch length.) Secure the S with fine wire or rubber bands. Carefully put a small amount of clay around the open end of the test tube. Then insert the clay and the S tubing into the opening of the tube. Be sure to keep the tube standing upright, and do not allow its contents to touch the clay or the plastic tubing.

5. Set the rack with all three tubes in an incubator. Incubate at 35° C.

6. Record observations every 24 hours for a period of three weeks.

Procedure Six: Applying the Pure-Culture Technique

When cultures are made from surfaces or from cultures which have been purposely mixed, the usual procedure is to begin the culture in a liquid or broth medium. (See Procedure Three -- "Inoculating

Liquid Broth.") The following sequential techniques are used to isolate a single species of organism:

1. With the inoculating needle, take a loopful of liquid broth from the tube that contains the organisms. Streak a sterile agar plate, following the directions for streaking (Procedure Four). The plate is then inverted and incubated for 24 hours.

2. After 24 hours, the plate is examined for colony formations. Only those colonies that have dropped off during the sweeping motions toward the end of the plate are selected for transfer. They must be isolated. They must be sufficiently separated from other colonies that they can be picked up from the surface of the solid agar and transferred again to the liquid medium. (Usually, 24 hours constitute sufficient growth time to produce adequate colonies for pure culture.)

3. Use a separate tube of broth for each colony that is morphologically different. Each tube of liquid broth now contains a pure culture of just one kind of organism.

4. The pure culture in each tube must be transferred to a new tube of broth medium at least once a week to keep the organisms young and actively growing.

Procedure Seven: Preparing Serial Dilutions for Studying Soil Bacteria

1. Take five tubes, each containing 10 cc of sterile water.

2. Measure 1 gram of the soil of your choice.

3. Dissolve the soil in 250 cc of water.

4. Filter through appropriate filter paper.

5. Inoculate tube #1 with one loopful of soil which has been mixed with 250 cc of water.

6. Flame the needle and take one loopful of the first mixture (tube #1 of the broth), and transfer to tube #2. Mix thoroughly.

7. Flame the needle. Transfer one loopful from tube # 2 to tube # 3. Mix thoroughly.

8. Flame the needle. Transfer one loopful of tube #4 to tube #5. Mix thoroughly.

9. Take five empty sterile petri dishes. Identify each dish with a number corresponding to each of the five tubes of water. Place one loopful of liquid from tube #1 in the bottom and at the center of

petri dish #1. Place one loopful of broth from tubes #2, #3, #4, and #5 into the corresponding petri dishes in the same way.

10. Melt one bottle of stock sterile agar. Cool to 50° C. Pour the agar, using the sterilization technique, into each of the five petri dishes. Mix thoroughly by swirling. Be sure that the agar is not too warm; it should not be warmer than 50° C.

11. Allow the mixture to harden. Invert the plates and put them in an incubator.

12. After 24 hours, you should have sufficient dilution of soil samples to allow individual soil colonies to form. There should be a gradation of growth from plate #1 to plate #5. Plate #1 should be almost too full of growth to allow any kind of individual colony study. Plate #5 should allow for easy counting of the number of distinct colonies.

Procedure Eight: Inducing and Studying Effects of Drying upon Organisms

1. A 24-hour culture of a single type of organism is obtained.

2. A single loopful of this 24-hour culture is placed in the center of each of five petri dishes.

3. Plate #1 has had warm, but not hot, agar poured into it; the agar is mixed and incubated without any drying.

4. The other plates are placed on a table so located in the room that the air is warm enough to allow drying to occur.

5. Plate #2 can have agar poured into it after 24 hours of drying. Invert the plate and incubate.

6. Pour agar into the third petri dish after three days of drying. If growth is obtained easily after this amount of time, extend the period of drying before pouring agar into the fourth and fifth plates. The drying time can be extended to seven, eight, nine, or ten days. What effect does drying have upon the growth of bacteria?

Procedure Nine: Applying Techniques of Staining

1. Preparation of a wet mount

The "wet mount" is used to study larger microorganisms, such as protozoans, yeasts, and some algae, as well as some molds. It is possible to observe protozoans if you use this procedure; these move rapidly. A wet mount can also be used to observe a colony of bacteria.

a. Take a clean glass slide. Insert a medicine dropper into the bottom layer of the jar containing the specimen to be examined.

b. Place a drop of water onto the middle of a clean slide.

c. Flame a wire needle. Touch the colony of bacteria to be examined and transfer the colony to the center of the drop of water on the slide. Gently mix the two.

d. Flame the inoculating needle to red-hot again. Then take a clean cover glass (also called "cover slip") by its edges and position it above the glass slide. Gently lower the cover glass over the droplet of water and bacteria in the center of the slide. Now observe the specimen under your microscope, at low power. A specimen prepared in this manner is called a wet mount. It is unstained.

e. When you have finished, be sure to deposit the slide and the cover glass in a jar of disinfectant provided for this experiment.

2. Methylene blue staining procedure

A relatively simple and inexpensive stain can be used to observe the morphology of individual organisms.

Materials: Aluminum broiler tray; slides; methylene blue stain; a small bottle (used to hold water for washing off the stain).

a. Take a clean glass slide which has been washed and dried in alcohol to remove all traces of grease. (This precautionary treatment should be given to all slides being used in bacteriological work.)

b. Place a drop of water on the center of the slide.

c. Flame a needle. Pick up a single colony from the surface of a solid medium – or a loopful from a liquid culture – and gently mix it in the drop of water. Spread out the tiny specimens so that the water covers at least twice the area it did when it was dropped onto the slide.

d. Be sure to flame the needle after mixing thoroughly.

e. Allow the slide to dry slowly. When it has dried, the residue (the "smear") takes on a slightly white appearance and can easily be seen on the glass slide.

f. Pass the slide through a flame (a gas flame or an alcohol burner flame), until the bottom of the slide is too warm to be held or touched comfortably with the fingers. This procedure is called "fixing" the slide and is necessary for *all* staining techniques. Fixing the slide prevents the bacteria from being swept away by the liquid from the stain or by the water that washes off the stain.

g. Cover the smear on the slide with methylene blue stain. Depending on the strength of the stain, allow it to stand on the smear from about 30 seconds to 1 minute.

h. Gently wash off the stain with water from a plastic wash bottle. The slide can be dried either by standing it on its end on a blotter or by placing it between two blotters and blotting it dry. When the slide is dry, place a drop of immersion oil on the smear. Put the slide on the microscope stage and gently lower the oil-immersion lens into the oil.

3. Gram's differential stain

This is the most complicated staining technique you will have to perform, and yet it is the most valuable in the study of microbiology.

Materials: Stain solution of crystal violet; a decolorizer; a solution of Gram's iodine; safranin; immersion oil; slides; a slide holder (such as a clothespin); an aluminum broiler tray to hold the slides; a wash bottle; a wire needle; and a culture.

a. Prepare the slide as before. Be sure that it contains no grease.

b. Place a drop of water on the center of the slide. Transfer a colony from the surface of a medium to the center of the slide. Mix thoroughly. Spread out over part of the slide and allow to dry. Fix the bacteria by passing the glass slide through a flame until the slide is very warm to the touch. Begin the staining procedure.

c. Flood the slide – or at least cover the smear – with a solution of crystal violet. Allow the stain to stand from 1 to 2 minutes. You will have to experiment with your own batch of crystal violet stain. Some stains are fairly strong; some are very weak.

d. Wash the slide gently with water to remove the purplish stain.

e. Cover the smear with Gram's iodine solution. Allow it to stand from 1 to 2 minutes. Wash off the iodine with water from the water bottle.

f. Hold the slide with a slide holder. Gently pour a small amount of decolorizing solution over the smear area. This solution should not be allowed to remain on the smear area for more than 15 to 20 seconds. Then wash the smear with water.

g. Now apply a 1 percent solution of safranin dye to the smear area. Allow the dye to remain from 1 to 2 minutes. Wash the stain off with water.

h. Allow the smear to drain, or blot it dry. Then apply a drop of immersion oil. Examine the slide with the oil-immersion lens.

4. A negative stain

This is a stain which can be used inexpensively and effectively to study organisms that do not take stains easily. All that is needed is a solution of India ink.

a. Put a small drop of India ink on one end of a clean slide. Place a small amount of the material to be stained in the center of the drop of India ink and mix well.

b. When the India ink and the material to be examined have been thoroughly mixed, take another glass slide that is clean and dry. Hold one end of it and place the other end in the center of the drop of the India ink mixture. The drop will now spread to both sides of the slide underneath the edge of the slide which you are holding with your fingers.

c. Now, gently push the slide which you are holding to the end of the slide that has the drop of India ink and the organisms. This action will cause the mixture containing the India ink and the organisms to be smeared evenly across the entire surface of the main slide.

d. Deposit the extra slide in the disinfectant container.

e. Allow the smear-film on the India ink slide to dry. You can now examine this film under the oil-immersion lens. You will find that the microorganisms will stand out white and that the background will be black. The organisms will not be distorted because they have not been fixed and have not been dried or subjected to chemical stains. The morphology of the individual organisms is thus easier to study.

Procedure Ten: Applying Techniques of Sterilization

1. For liquid media in tubes without sugar, maintain pressure in the pressure cooker at 15 pounds for 15 minutes. Trypticase soy agar for pour plates should be pressured 20 minutes at 15 pounds.

2. For glassware, slides, and culture tubes – all with organisms to be destroyed, maintain pressure for 15 minutes at 15 pounds. Be sure that the caps of tubes are loose before raising the pressure. Never pour live cultures down the sink.

3. Media containing carbohydrates which would be coagulated by the high temperatures of a pressure cooker may be sterilized by placing the tubes in water-baths or in hot-air ovens at a temperature between 60° and 70° C. for one hour for five consecutive days.

4. Boiling tubes and slides for 20 minutes is an alternate method for sterilizing glassware before disposal or reuse.

5. Prepare one or more of the following solutions in order to sterilize tabletops after use or to hold slides which have already been used: (a) 5 percent phenol solution; (b) halimide solution – 1 oz./ 1 gal. H_2O; or (c) 0.1 percent of trichloride of iodine (ICl_3).

RESOURCES AND REFERENCES

This part of the guide on microbiology contains (1) a list of supplies that would be needed for experiments and projects of the type suggested in sections I, II, and III; (2) information regarding suppliers of materials and equipment; (3) a reading list of books recommended for gifted pupils in grades four through six; and (4) a brief list of professional references.

Suppliers and Suppliers

The list of supplies is not an exhaustive one. The teacher, the learners, and other interested persons may suggest additional useful items and may also be able to bring to light some information about suppliers and sources of supply not mentioned here.

Supplies

Agar-agar powder
Alcohol lamp
Aluminum-foil broiler trays
Balance for measuring weight
Broth -- specially prepared for culturing experiments
Clay
Clothespins
Cotton
Disinfectant solution
Distilled water
Filter paper
Flasks
Funnels
Halimide solution
Hand lenses
Immersion oil
Incubator
India ink
Inoculating needles (platinum wire)
Marking crayons
Medicine droppers
Microscope with oil-immersion lens
Notebooks
Nutrient agar powder

Nutrients, such as dextrose, levulose, and glucose
Petri dishes
Plastic sacks
Plastic wire
Pressure cooker
Slides and covers
Soaps
Soil in sufficient quantities
Stains – methylene blue, crystal violet, Gram's decolorizer, Gram's iodine, safranin
Sterile cotton-tipped applicators
Sterile sticks for applicators
Test-tube rack
Test tubes with screw caps – 15 ml capacity
Trypticase soy agar
Trypticase soy broth
Tubing – plastic
Wash bottles with screw caps – 150 ml capacity

Suppliers

Science kits containing most of the equipment necessary for microbiological study, research, and experimentation are available from a number of firms in California and elsewhere in the nation. These kits are usually accompanied by informative guides or manuals.

The Scientific Products Division of the American Hospital Supply Corporation is willing to send out any amount of materials, C.O.D., from either of the following offices on the same day that the order is received:

17111 Red Hill Ave.
Santa Ana, Calif.
Telephone: 714-540-5320

150 Jefferson Dr.
Menlo Park, Calif.
Telephone: 415-323-7741

Supplies may be obtained also from laboratories in local clinics or hospitals.

Selected References

Good reading can help gifted children enormously as they make their way through this unit. Not only will they acquire more knowledge and insight, but also will they discover – by learning about other people and knowing how hard these people have worked to improve the human condition – that the world of nature is indeed wonderful and amazing.

By the same token, it is quite likely that the teacher will make his instruction a more fulfilling experience if he shores up and enriches his stock of knowledge by doing some additional reading and

research on a professional level. Obviously, the teacher who finds microbiology unfamiliar ground to walk on will benefit the most.

Reading List for Pupils

Beeler, Nelson F., and Franklyn M. Branley. *Experiments with a Microscope.* New York: Thomas Y. Crowell Company, 1957.

Beiser, Arthur. *Guide to the Microscope.* New York: E. P. Dutton & Co., Inc., 1957.

Berger, Melvin. *Triumphs of Modern Science.* New York: McGraw-Hill Book Company, 1964.

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Cosgrove, Margaret. *Wonders Under a Microscope.* New York: Dodd, Mead & Company, 1959.

De Kruif, Paul. *Microbe Hunters* (Text edition). Edited by Harry G. Grover. New York: Harcourt, Brace & World, Inc., 1939.

Dubos, René J. *The Unseen World.* New York: Rockefeller Institute Press, 1962.

Elwell, Felicia R., and J. M. Richardson. *Science and the Doctor.* New York: S. G. Phillips, Inc., 1959.

Grant, Madeleine P. *Louis Pasteur: Fighting Hero of Science.* New York: McGraw-Hill Book Company, 1959.

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Kavaler, Lucy. *Wonders of Algae.* New York: John Day Company, 1961.

Knight, David C. *Robert Koch: Father of Bacteriology.* New York: Franklin Watts, Inc., 1961.

Kohn, Bernice. *Our Tiny Servants: Molds and Yeasts.* New York: Prentice-Hall, Inc. 1962.

Leeuwenhoek, Antony van. *Antony van Leeuwenhoek and His Little Animals.* Translated and edited by Clifford Dobell. New York: Dover Publications, Inc., 1962.

Lewis, Lucia Z. *First Book of Microbes.* New York: Franklin Watts, Inc., 1955.

Ludovici, Laurence J. *The World of the Microscope.* New York: G. P. Putnam's Sons, 1959.

Schatz, Albert, and Sarah R. Riedman. *Story of Microbes.* New York: Harper & Row Publishers, Inc., 1952.

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Williams, Greer. *Virus Hunters.* New York: Alfred A. Knopf, Inc., 1959.

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Zinsser, Hans. *Rats, Lice, and History*. Boston: Printed and published for the Atlantic Monthly Press by Little, Brown & Co., 1935. (Paperback edition – New York: Bantam Books, Inc., 1960.)

Professional References

Bergey, David H. *Bergey's Manual of Determinative Bacteriology* (Seventh edition). Edited by Robert S. Breed and Others. Society of American Bacteriologists. Baltimore, Md.: Williams & Wilkins Company, 1957.

Conant, Norman F., and Others. *Manual of Clinical Mycology* (Second edition). Philadelphia: W. B. Saunders Co., 1954.

Hone, Elizabeth B., Alexander Joseph, and Edward Victor. *Teaching Elementary Science: A Sourcebook for Elementary Science*. Under the general editorship of Paul F. Brandwein. New York: Harcourt, Brace & World, Inc., 1962.

Salle, Anthony J. *Fundamental Principles of Bacteriology* (Fifth edition). New York: McGraw-Hill Book Company, 1967.

Taxonomy of Educational Objectives: The Classification of Educational Goals. In two volumes. *Handbook I: Cognitive Domain;* edited by Benjamin S. Bloom and Others, 1956. *Handbook II: Affective Domain;* edited by D. R. Krathwohl and Others, 1964. New York: David McKay Co., Inc.

Zinsser, Hans. *Microbiology* (Thirteenth edition). Edited by David T. Smith and Others. New York: Appleton-Century-Crofts, 1960.

Addendum

FURTHER STUDY OF MICROBIOLOGY

A second unit on microbiology can be made available to those gifted learners who have found the activities in the present guide so much to their liking that they want to learn more about this special branch of biology.

It should be made clear that no pupil is obliged to take on the additional coursework; nor should any pupil feel that he needs to "master" the content of this guide before going further. The decision of each child should be based on his own interests and desires.

The material that follows includes brief guidelines for moving from the present unit on nonpathogenic organisms into a study of disease-producing organisms. The purpose of this material is to suggest to the teacher one of the possible directions that can be taken for the continuation of research and experimentation in the field of microbiology.

Concepts and Objectives

A major concept and three objectives are presented here. Other concepts and objectives can be included in the plans that are formulated before the new unit begins.

Concept

The existence of microorganisms affects human life.

Objectives

1. *The learner will culture specimens of organic materials in varying states of decay and decomposition.*
2. *The learner will rediscover and apply the techniques of sterilization.*
3. *The learner will understand the importance of personal health habits and the rationale of disease control.*

General Remarks

Some organisms found in the air, in the soil, and in the water are helpful to man when they perform certain tasks of garbage disposal and create certain products that are used by the human community as foods, salutary drugs, and medicines.

On the other hand, disease-producing organisms also exist, and it is highly important that man knows how to cope with them. Albeit, rather specific requirements must be met before they can grow outside their human host.

In order to induce pathogenic microorganisms to grow in a petri dish, (1) special nutritional supplements must be added; (2) sometimes a special atmosphere, reduced in oxygen, must be arranged; and (3) a slightly higher temperature must be maintained. Disease-producing organisms will not be found in the usual cultures of air, soil, or water. They must be cultured from excrements or from something that has had direct contact with a person stricken by a particular disease.

A number of experiments can be carried out by the pupils and guided by the teacher. For example, if the children wish to observe growth that can occur in the wake of a cough or a sneeze, they should cough or sneeze into a culture plate. The plate should then be sealed tight. Observations should be made only through the transparent plastic top; this precaution is necessary because some of the organisms might become virulent and infectious and of course must not be allowed to escape. After making notes of what they have observed, the pupils should report their findings to the class. The teacher and the learners can take part in an overall class discussion, and the activity can be supplemented by audiovisual aids and special research.

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